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Characterization of a New Enterococcal Gene, satG, Encoding a Putative Acetyltransferase Conferring Resistance to Streptogramin A Compounds

Streptogramin antibiotics are mixtures of two chemically unrelated A and B compounds that act synergistically in vivo against gram-positive pathogens, such as staphylococci, streptococci, and enterococci (8, 11). Resistance against B compounds is very widespread among enterococci and is mediated via the emB gene cluster (e.g., on Tn917) that confers macrolide-lincosamide-streptogramin B resistance (7). The synergistic mixture of streptogramins A and B overcomes resistance to B compounds but is inactive in resistance to A compounds. The only known resistance mechanism against streptogramin A compounds in enterococci is mediated by the streptogramin acetyltransferase SatA (9). Enterococcus faecium isolates with satA-mediated resistance have been found in samples of human and animal origins, indicating a possible spread of resistance genes or resistant bacteria among different ecosystems (10).

We isolated a quinupristin-dalfopristin-resistant E. faecium UW1965 from a sewage treatment plant in Germany. The resistance determinant was transferred to a susceptible recipient, producing the transconjugant UW1965K1. UW1965K1 is resistant to quinupristin-dalfopristin (MIC ≥ 16 μg/ml) and virginiamycin M (A compound; MIC, 16 μg/ml), whereas the

MIC of each antibiotic for the recipient was 1 μg/ml. PCR amplification for the satA gene was negative.

In staphylococci, resistance to streptogramin A compounds is mediated by two mechanisms: (i) acetylation of the streptogramin A via acetyltransferases (Vat, VatB, and VatC [1-3]) and (ii) efflux due to an ABC transporter (Vga and VgaB [4, 5]). PCR amplification for the vat, vatB, vatC, and vga genes failed to produce any product. The putative protein sequences of the known streptogramin acetyltransferases in staphylococci and enterococci contain three conserved motifs (2). Corresponding primers, satl and satl, have been made, producing a 144- to 147-bp fragment for vat, satA, and vatB (2). PCR performed with these primers resulted in a ca. 150-bp fragment for UW1965K1. A digoxigenin-labelled probe of the amplified fragment was prepared, hybridizing with a 5.5-kbp fragment of EcoRI-digested plasmid DNA from the transconjugant. The corresponding plasmid fragment was cloned into pUC18 and sequenced.

The resulting DNA sequence (Fig. 1) did not show significant identity with other gene sequences from GenBank on the DNA level (6). One suitable open reading frame (ORF) was found, giving rise to a putative 214-amino-acid (214-aa) pro-

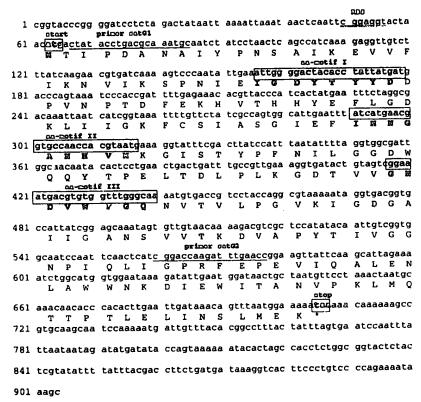


FIG. 1. A 904-bp sequence located on the 5.5-kbp cloned fragment in pUC18 (GenBank accession no. AF139725). The ORF begins at nucleotide 63 with an ATG start codon preceding a putative ribosomal binding site (RBS) (double-underlined) at positions 50 to 57. The predicted gene sequence encodes a protein of 214 as which shows significant homology with other streptogramin acetyltransferases (as motifs 1, 11, 111; see also Fig. 2). The locations of the primers satG1 and satG2, specific only for the satG sequence, are underlined (plus strand).

Motif I M----TIPDANAIYPNSAIKEVVFIKNVI-KSPNIEIGDYTYYDDPVNPTDPEKHVTHHYEFLGDKLI 63 SatG: 1 MK----YGPDPNSIYPHEBIKSVCFIKNTI-TWPNIIWGDYXYYDDVNGAEKPEEHVTHHYEPRGDKLV 64 VatB: 1 MKWQNQQGPNPEEIYPIBGNKHVQPIKPSI-TKPNILVBUYBYYDSK-DGESPESQVLYHYELIGDKLI 67 M-----GPNPWGMYPIBGNKSVQPIKPILEKLENVEVGUYBYYDSK-NGETPDKQILYHYPILNDKLK 62 VatC: MALANDHGPDPENILPIKGNENLQPIKPTI-TNENILWGDYSYYDSKRGES-PEDQVLYHYEVIGDKLI Vat: Cotif II IGKPCSIASGIBPTT ZANTIN KGISTYPPNILGGDMQQYTPEL-TDLPLKGDTVVGEBVCDGQNV 128 IGKPCATAPGIEPTS ZANTIN NSITTYPPNIKGNGWEKATPSL-EDLPPKGDTVVGEBVCDGQNV 129 SatG: 64 VatB: 65 LGKPCSIGPOTTPILL ON THE DO-STPPPNLPGNGWEKHTPTL-BDLPYKGNTBIG DWINGROV 131 VatC: 68 IGKPCSIGPGVTIII: CV FILLIDG-STYPPNLPGNGWERHMPKL-DQLPIKGDTII CEDWIIGKDV SatA: 63 IGRPCSIGPGTTPIE: 2011 DEL DG-STYPPHLPRMGWEKYMPSL-KDLPLKGDIBIGEDVIIGRDV 131 Vat: 68 Satg: 129 TVLPGVKIGDGAIIGANSVVTKDVAPYTIVGGNPIQLIGPRPEPEVIQALENLAW 183 130 TVMPGIGIGDGAIVAANSVVTKDVPPYRIIGGNPSRIIKKRPEDELIDYLLQIKM 184 132 TIMPOVKIGNGAIIAARSVVTKNVDPYSVVGGNPSRLIKIRFSKEKIAALLKVRW 186 127 VIMPOVKIGDGAIVAANSVVVKDIAPYMLAGGNPANEIKGRPDODTINQLLDIKW 181 132 TIMPOVKIGDGAIIAARAVVTKNVAPYSIVGGNPLKFIRKRPSDGVIEEWLALQW 186 SatA: Sato: 184 WINKDIEWITANVPKLMQTTPTLELINSLMEK 214 VALE: 185 WDMSAQKIFSKLETICSS--DLEKIKSIRD 212 VALC: 187 WDLEIETINENI 188 Sata: 182 WDMFIDINENIDKILDNSIIREVI 206 VAL: 187 WNLDMKIINENIP 199

FIG. 2. Alignment of amino acid sequences of acetyltransferases from staphylococci and enterococci (1-3, 9) conferring resistance to streptogramin A antibiotics. Identical residues are indicated by asterisks. Highly conserved regions in different streptogramin A acetyltransferases—motifs 1, 11, and 111—are holdfaced. Primers sail and sail have been designed on the basis of the corresponding nucleotide sequences in motifs 11 and 111 (2).

tein. A comparison of amino acid similarities indicated rather significant homology between streptogramin acetyltransferases and the new putative acetyltransferase, designated SatG (Fig. 2). Based on the sequence for satG, two primers, satG1 and satG2, have been designed. Preliminary results of a search for streptogramin-resistant enterococci (E. faecium, E. hirae, and E. durans) revealed the existence of the satG gene in 9 of 23 isolates from sewage, 6 of 24 isolates from broiler samples, and all 17 isolates from poultry manure. Of 62 quinupristin-dalfo-pristin-resistant E. faecium (QDREF) isolates from hospitals in Germany, 9 were positive for satG. The high number of satG QDREF isolates from poultry meat and manure may be due to selection of these bacteria by use of virginiamycin as a feed additive, and spread of the resistance via the food chain to humans is very likely. This hypothesis is being investigated.

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